Atlantic salmon:
Natural history, aquaculture, genetic improvement

- and -

Ecological risk assessment for transgenic fish

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Atlantic salmon

Salmo salar Linneaus 1758
Salmo – Latin, "salmon"
salio – Latin, "to leap"

Taxonomic classification:

- Phylum Choradata
- Subphylum Vertebrata
- Class Actinopterygii
- Order Salmoniformes
- Family Salmonidae
- Genus *Salmo*
- Species *salar*

Morphological description:

- Body trout-like, elongate
- Somewhat compressed laterally
- Mouth terminal, large, maxillary extending to posterior margin of pupil
- Male males develop pounced hook, or "skype", on lower jaw
- Teeth developed on premaxillary, maxillary, and dentary bones of jaw
- Gill-rakers 15-20
- Spines absent
- Adipose fin present
- Scales cycloid
- Pyloric caecae 40-74
- Vertebrae 58-61
- …

Atlantic salmon

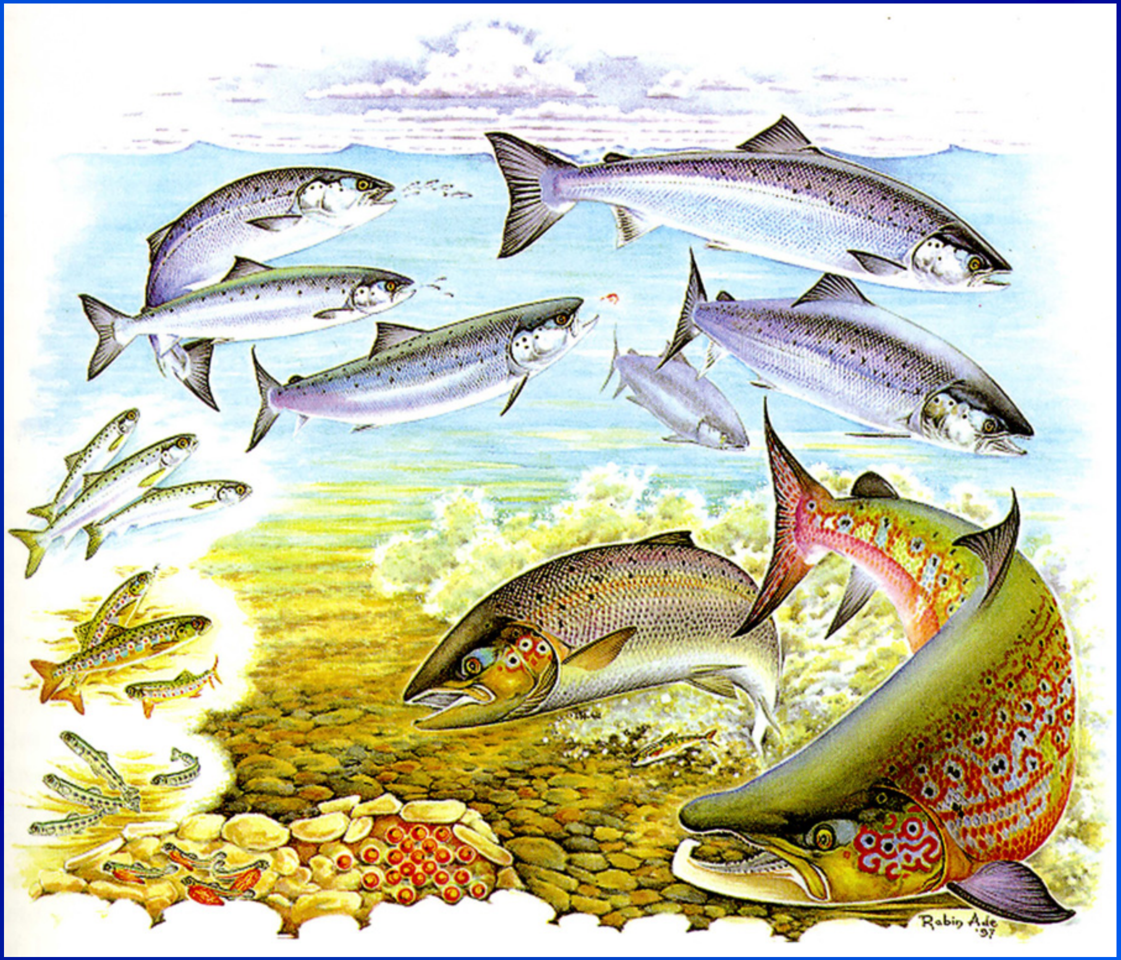
Salmon image incised in stone by Pictish people 1000 YA, Scotland (Verspoor, 2007)

An iconic species, the "King of Fish":

- •Mentioned in the Magna Carta
- •A popular sportfish
- •Thousands of scientific papers on biology and management
- •Hundreds of books
- •Still, many aspects of its biology, genetics, and ecological adaptations remain poorly characterized

Life cycle of Atlantic salmon

(anadromous life history; adfluvial and non-anadromous life histories also exist)



Post-smolts

Smolts

Parr

Fry

Migratory adults

Spawning adults

Kelts – salmon after spawning – not shown

Alevins

Eggs

(R. Ade, Atlantic Salmon Trust)

Life cycle of Atlantic salmon

Atlantic salmon males exhibit alternative reproductive strategies:

•*Precocious parr* do not migrate, mature young and small, and sneak fertilizations, sometimes with great success (11-65% of fertilizations in some populations)

•*Grilse* migrate to sea for one year, mature medium-sized, and sneak fertilizations

•*Parental males* migrate to sea, mature large, defend territory, and court females

•Expression of early maturation is related to growth rate

Adaptation and differentiation of salmon populations

- Salmon exhibit a high degree of homing for spawning
- “Each river has its own peculiar race of fish, and each race finds its own river with most perfect decision.”

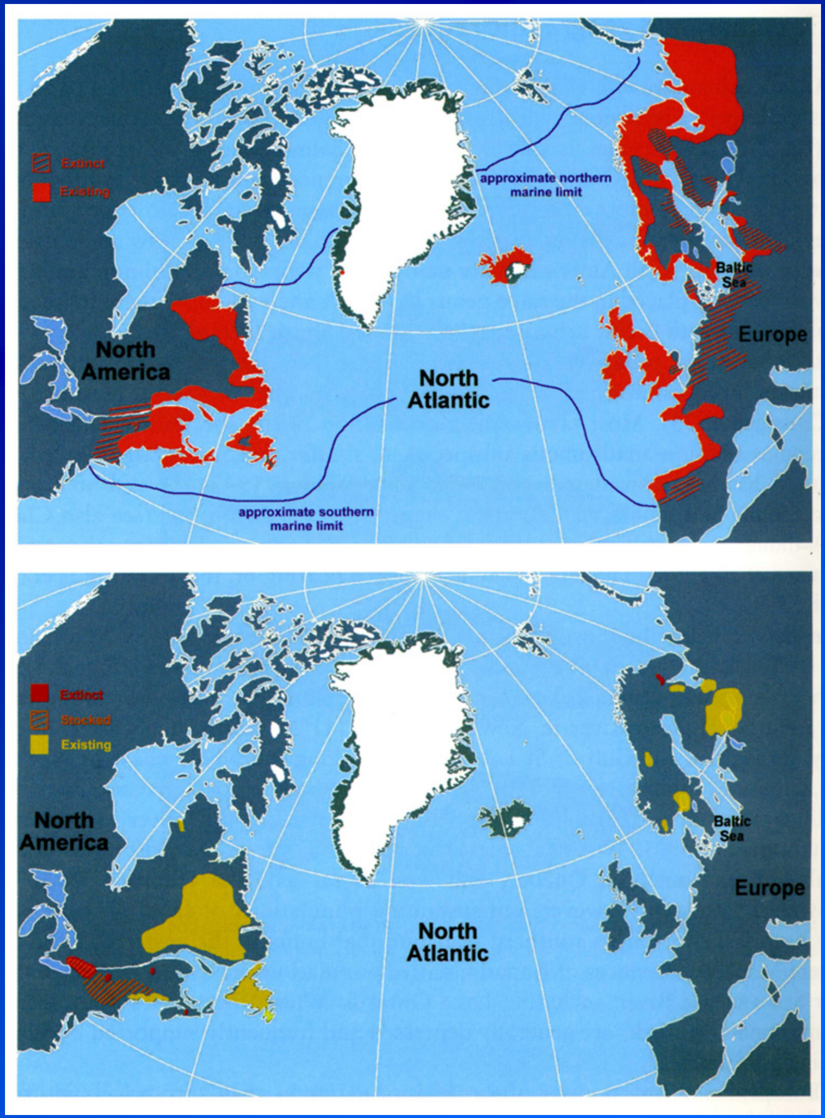
  – Andrew Young (1854) *The Natural History and Habits of Salmon*

- Selective pressures upon life-history traits give rise to:
- Local adaptation
- Genetic differentiation among populations

Range of Atlantic salmon

- Broad range:
- Range of anadromous (migratory) stocks
- Range of non-anadromous (non-migratory) stocks

- Atlantic salmon has declined precipitously through much of the range



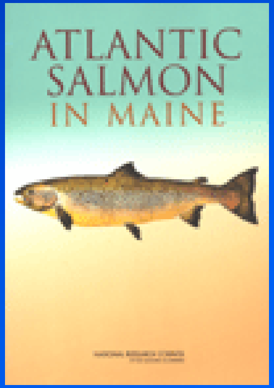
(Webb et al. 2007)

Conservation status of Atlantic salmon

- Listed on IUCN Red List as “Least Concern”
- International conservation effort coordinated by North Atlantic Salmon Conservation Organization

- Inner Bay of Fundy populations listed as Endangered by Canada

- Atlantic salmon now extinct in 84% of New England rivers once supporting them
- Atlantic salmon in rivers of Maine listed as endangered under the U.S. Endangered Species Act in 2000
- A Recovery Plan for Gulf of Maine Atlantic salmon has been developed and adopted



Aquaculture of Atlantic salmon

Aquaculture yields 69% of global salmon production (FAO 2008), and almost all Atlantic salmon consumed

Salmon culture:
Overview of the production cycle

Stripping eggs from female broodstock

Freshwater culture: 12-18 months

- Spawning of broodstock
- Hatchery
- Nursery – onshore
- Smoltification

Advanced parr and smolt

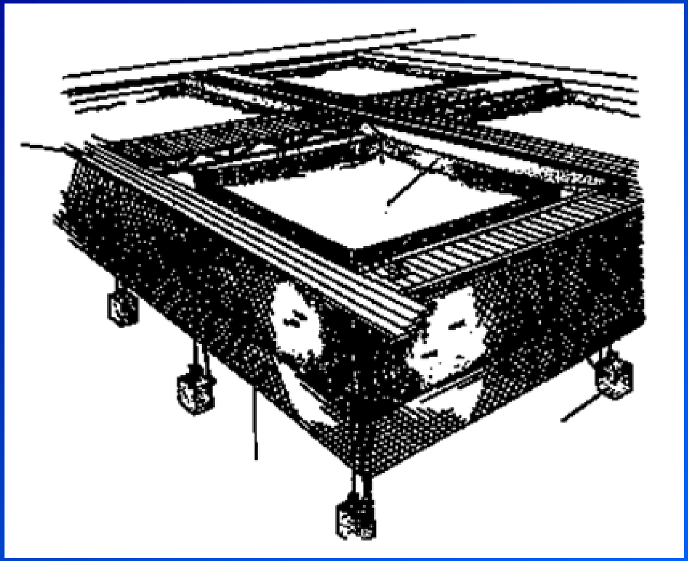
On-shore tank system

Salmon culture:
Overview of the production cycle

Grow-out 18-24 months (32 for broodstock)

- Marine net-pens
- Commercially proven

Marine net-pen



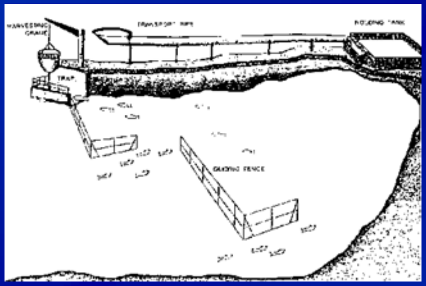
Drawing of net-pen showing supporting infrastructure

Salmon culture:
Overview of the production cycle

Other grow-out systems:

•Not commercially proven; pose different production characteristics

- Ocean ranching
- Land-based saltwater systems
- Sunken cage systems
- Recirculating aquaculture systems





Salmon culture:
Overview of the production cycle

Harvest and processing

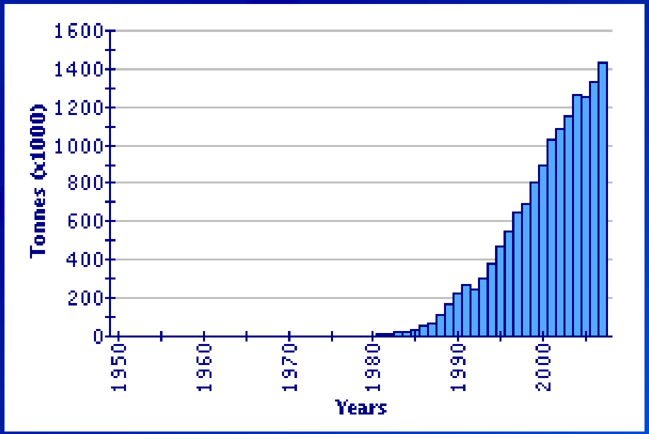

Harvest using a fish pump


Process line for filleted product

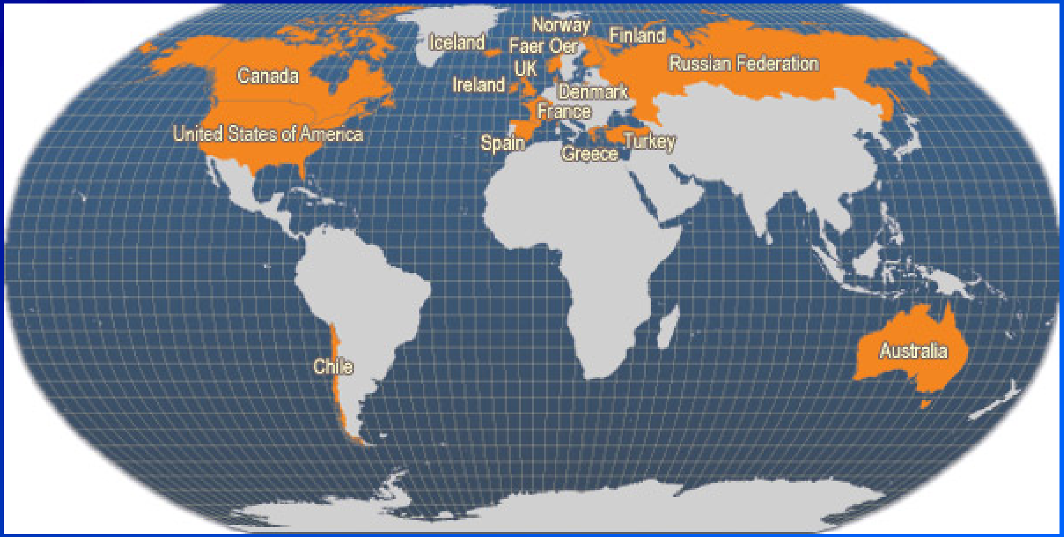
Atlantic salmon production

Global production 1,500,000 metric tons

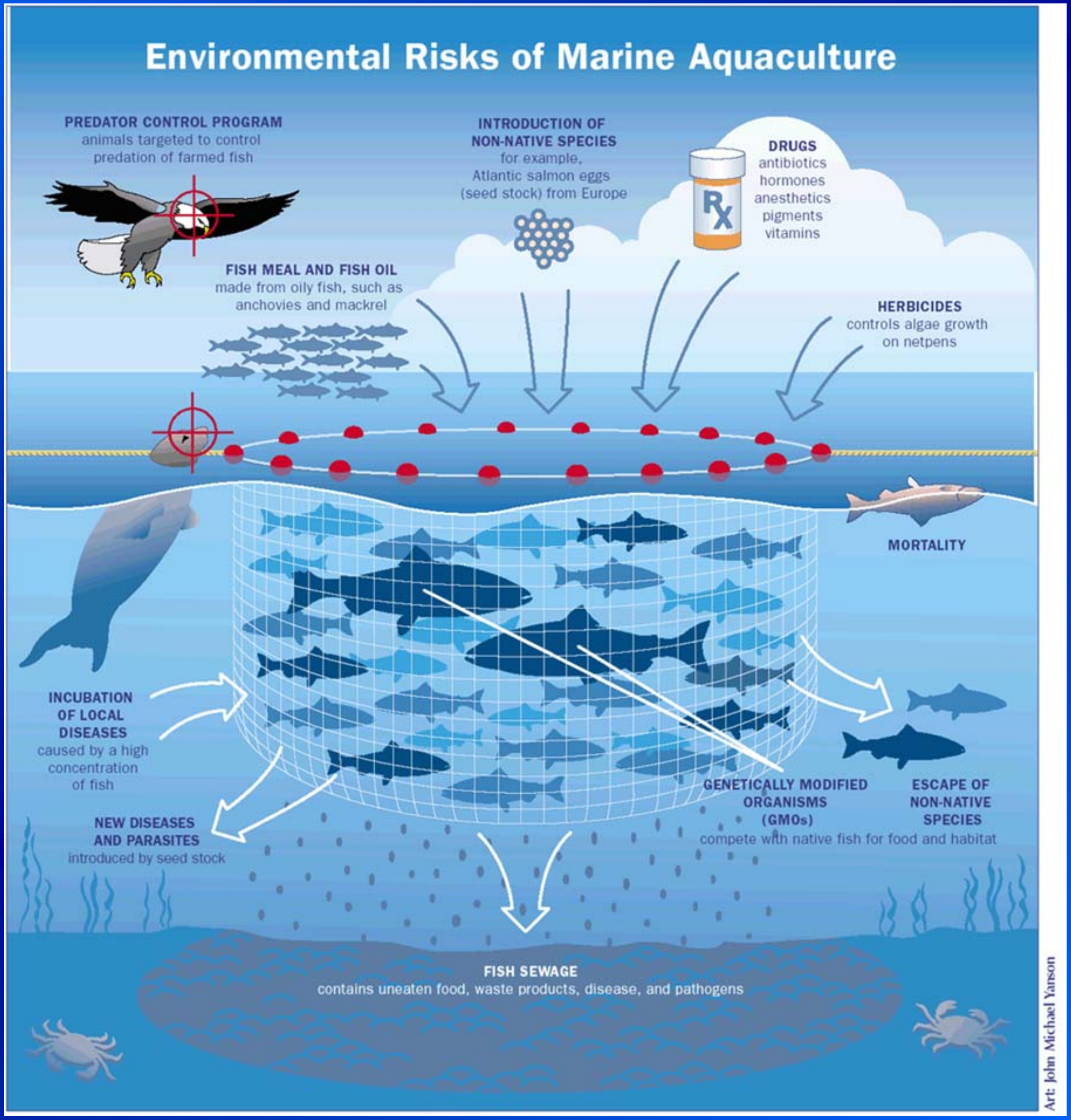
- •Leading producers:
- •Chile
- •Norway
- •Scotland
- •Canada
- •Faroe Islands
- •United States
- •Russia
- •Australia (Tasmania)
- •…



(FAO data)



Atlantic salmon production poses ecological impacts



Atlantic salmon breeding

Classical selective breeding:

- Noteworthy breeding programs:
- AKVAFORSK (Norwegian government – private sector collaboration)
- Since 1970, subsequently privatized as AquaGen and SalmoBreed
- Initially used mass selection for growth; subsequently used index-based within- and between-family selection for growth, age-at-maturity, disease resistance and flesh quality traits
- Salmon grow twice as fast as wild salmon and require 25% less feed
- Vertically integrated producers (1970s and 1980s) - their salmon somewhat less high-performance than the AKVAFORSK salmon
- USDA-ARS National Coldwater Aquaculture Center in Maine (2000s)
- Virtually all producers use selectively-bred stock

Atlantic salmon breeding

Biotechnology: All-female production

- Precludes precocious maturation of males
- Achieved by creating and breeding XX-neomales
- In commercial use

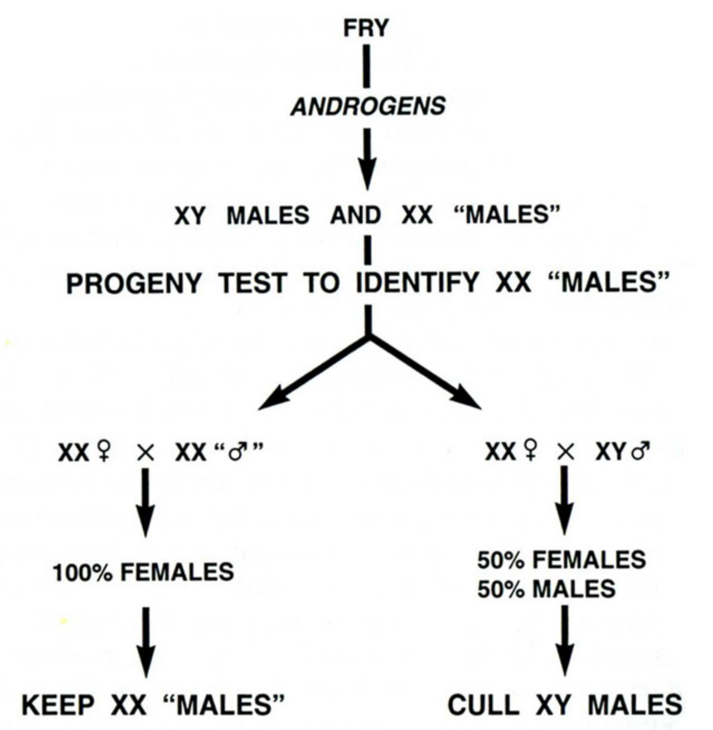


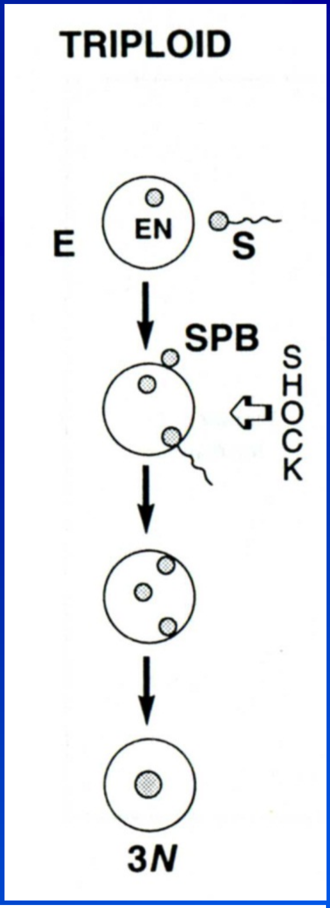
Figure 5.3 Schematic diagram of the protocol needed to create sex-reversed XX "male" broodstock that are capable of producing all-female populations for species with the XY sex-determining system.

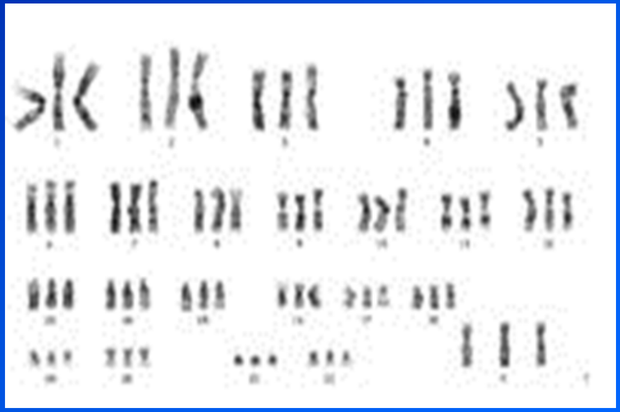
(Tave 1993)

Atlantic salmon breeding

<u>Biotechnology</u>: Triploidy induction

- Induces sterility
- Achieved by blocking last step of meiosis (extrusion of second polar body, SPB)
- In commercial use
- Males may mature, even if sterile; hence, production of triploid females preferable

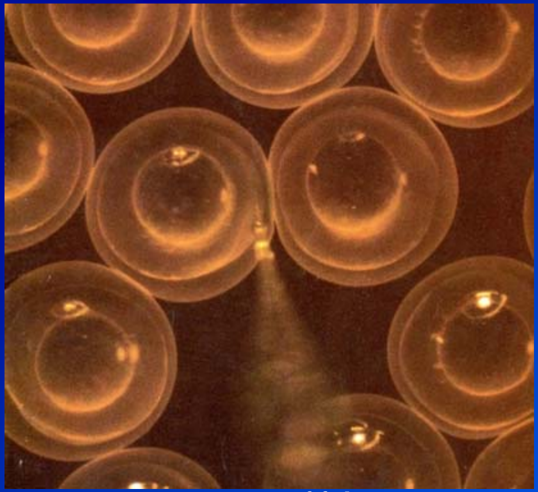




Atlantic salmon breeding

<u>Biotechnology</u>: Gene transfer

- <u>Anti-freeze polypeptide</u>: Aimed at protecting salmon in super-cooled water from freezing upon contact with nucleating ice crystals
- Insufficient freeze resistance achieved
- <u>Growth hormone</u>: 4-6x growth rate enhancement early in life, 10-20% improvement in FCR
- Prospect of shorter production time, reduced costs, improved efficiency and profitability



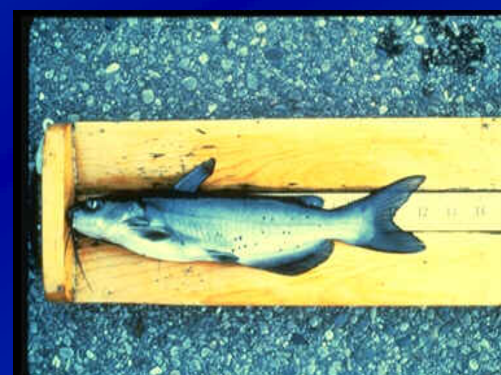
Microinjection of fish eggs

AquaBounty Atlantic salmon

A large international effort aims at developing transgenic aquatic organisms

Fishes:

Zebrafish
Medaka
Grass carp
Common carp
Goldfish
Wuchang fish
Giant loach
Northern pike
Rainbow trout
Coho salmon
Atlantic salmon
Arctic charr
Mummichog
Striped bass
Largemouth bass
Walleye
Nile and hybrid
   tilapias
Cutthroat trout
Catla
Rohu
Mrigal
Channel catfish
Indian catfish



Crustaceans:

Crayfish
Pacific white shrimp
Brine Shrimp
Tiger shrimp
Giant freshwater prawn
Kuruma prawn

Mollusks:

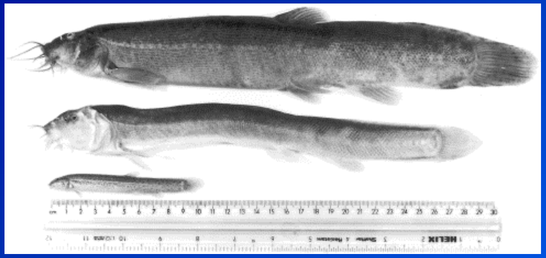
Pacific oyster
Eastern oyster
Blue mussel
Dwarf surfclam
Red abalone
Japanese abalone
Pearl oyster

Growth hormone-transgenic fishes developed for potential use in aquaculture:

Esocidae
- Northern pike

Salmonidae
- Atlantic salmon
- Coho salmon
- Chinook salmon
- Rainbow trout
- Cutthroat trout
- Arctic charr

Cyprinidae
- Goldfish
- Common carp
- Catla
- Rohu
- Mrigal



Cobitidae
- Mud loach

Ictaluridae
- Channel catfish

Heteropneustidae
- Indian catfish

Cichlidae
- Nile tilapia
- Hybrid tilapia

Percidae
- Walleye

A large international effort aims at developing transgenic aquatic organisms

Transgenes:

Reporter genes

Growth hormone
Antifreeze polypeptide
Interferon
Cecropin
Lactoferrin
Phytase
Carbohydrate metabolism
Vitamin C metabolism
GnRH antisense
Aromatase antisense

Human clotting factor VII
Insulin

Reporter genes for contaminants

Countries:

United States
Canada
Cuba
United Kingdom
France
Norway
China
Japan
Korea
India
Israel

Environmental safety of transgenic aquatic organisms

- Aquaculture production goes forward in a range of culture systems offering different degrees of confinement
- Escape from production facilities more or less likely…
- Could interbreeding with wild populations pose genetic and evolutionary harms to receiving populations?
- Could heightened predation, competition, or other processes pose ecological harms to receiving ecosystems?

How to approach defensible risk assessment and risk management for transgenic fishes?

I'll show:

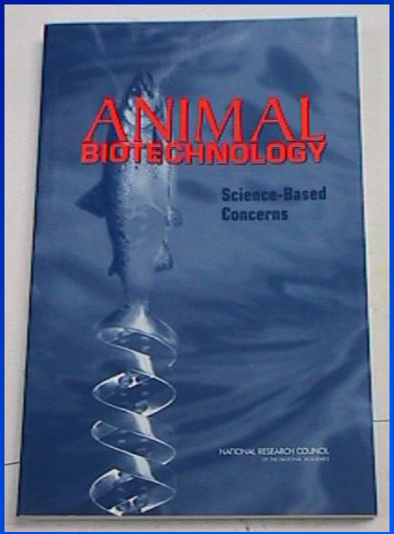
- Framework of risk assessment principles
- Supporting examples from empirical literature

Risk assessment framework

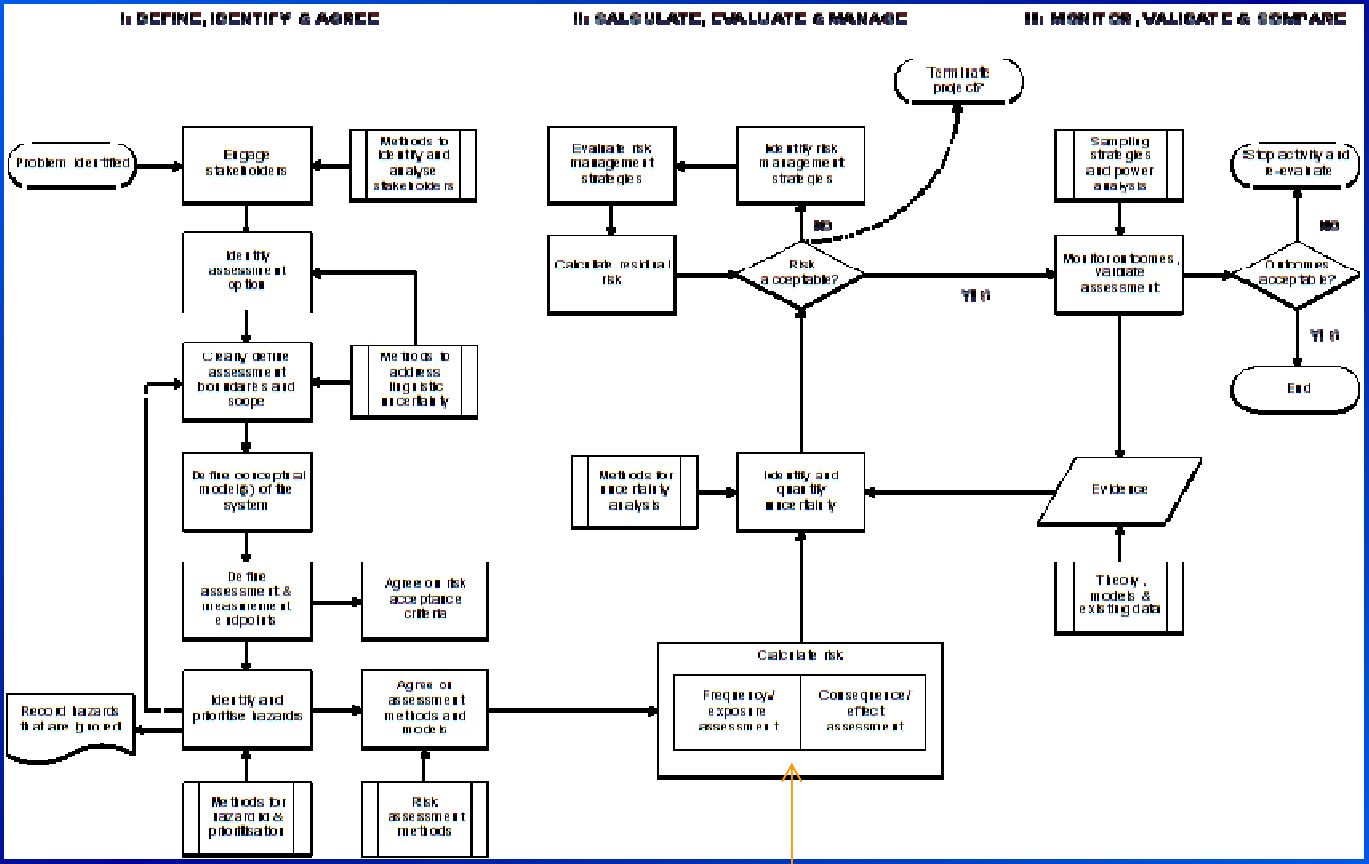
- Identify potential *harms* - outcomes
- Identify *hazards* that might lead to harms – the transgenic stock
- Assess *probability of exposure* – likelihood of escape and persistence of transgenics in receiving ecosystem
- Assess *probability of harm given exposure*
- $R = P(E) \times P(H|E)$
- Risk is a *probability*

Ecological risk assessment for transgenic organisms

- Considered on a case-by-case basis:
- Host species
- Introduced genetic construct
- Integration event
- Receiving ecosystem

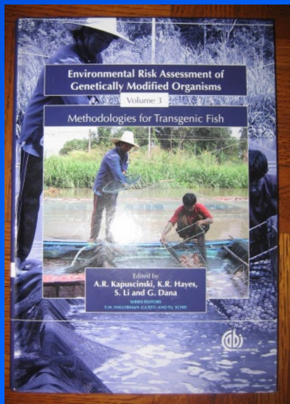


A risk assessment / risk management framework for transgenic fishes…



(Hayes et al. 2007)

Let’s focus on estimating risk associated with genetic and ecological processes

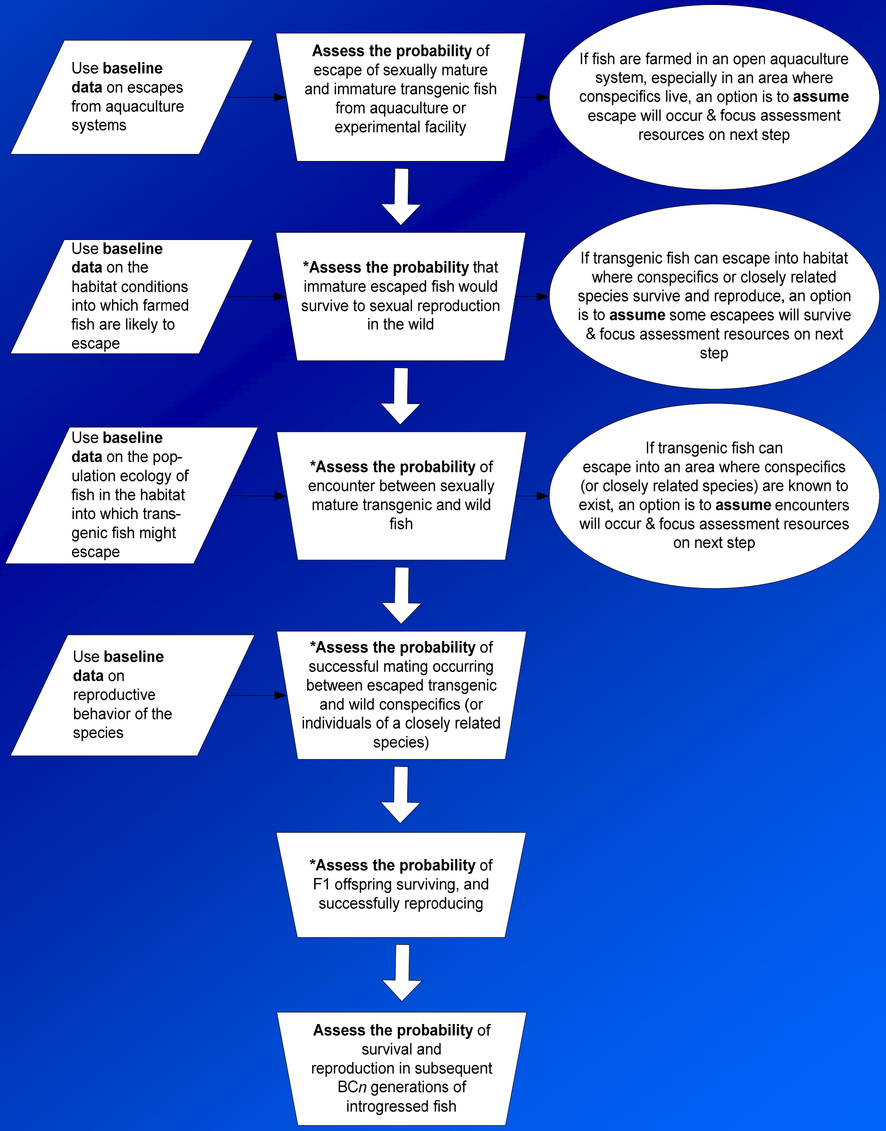


Could interbreeding of transgenic fish with wild populations pose genetic and evolutionary harms to receiving populations?

- Introgression is a risk *pathway*, but not a risk *endpoint* (i.e., not a harm in and of itself)
- Possible *harms*:
- Loss of (local) adaptation
- Reduced genetically effective population size (→ loss of genetic variation)
- In the extreme case, extinction of receiving population

Risk assessment

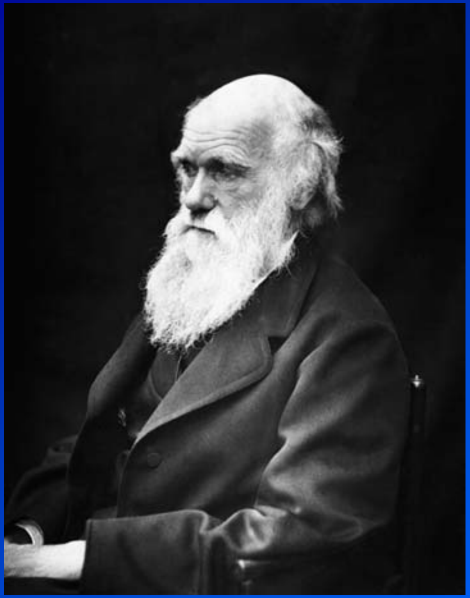
- Realization of harm requires occurrence of a chain of events
- Risk assessment is estimation of the likelihood of that chain of events occurring



(Kapuscinski et al. 2007)

Would the transgene be purged from population or would it persist?

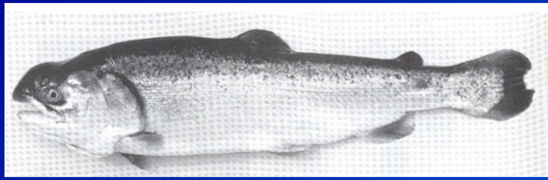
- Key unknown parameter is the *fitness* of transgenics and their offspring
- Darwinian fitness = ability to survive to reproductive maturity and to produce viable offspring



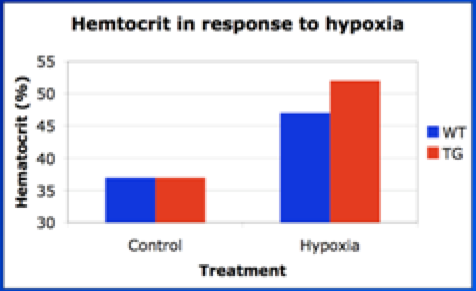
Charles Darwin

What is the *fitness* of transgenic individuals?

- Empirical observations of transgenics show:
  - Overgrowth of cartilage in some lines
  - 60% higher oxygen consumption rate
  - Lower critical oxygen concentration
  - Lower critical swimming speed
  - Higher willingness to risk exposure to predators
  - Lower viability of young
  - Decreased disease resistance
  - Decreased resistance to stress
  - Smoltification poorly tied to natural cues
  - …
- …suggest that transgenic individuals are *less* fit than non-transgenic individuals



(Devlin et al. 1995)



(Cnaani et al. unpublished)

What is the *fitness* of transgenic individuals?

- Negative impacts of expression of transgenes led some to suggest that transgenics pose no significant genetic risks
- However, empirical observations of *GH* transgenics show:
- Heightened growth rate
- Heightened food conversion efficiency
- Larger ultimate size – may confer mating advantage
- Increased osmoregulatory ability

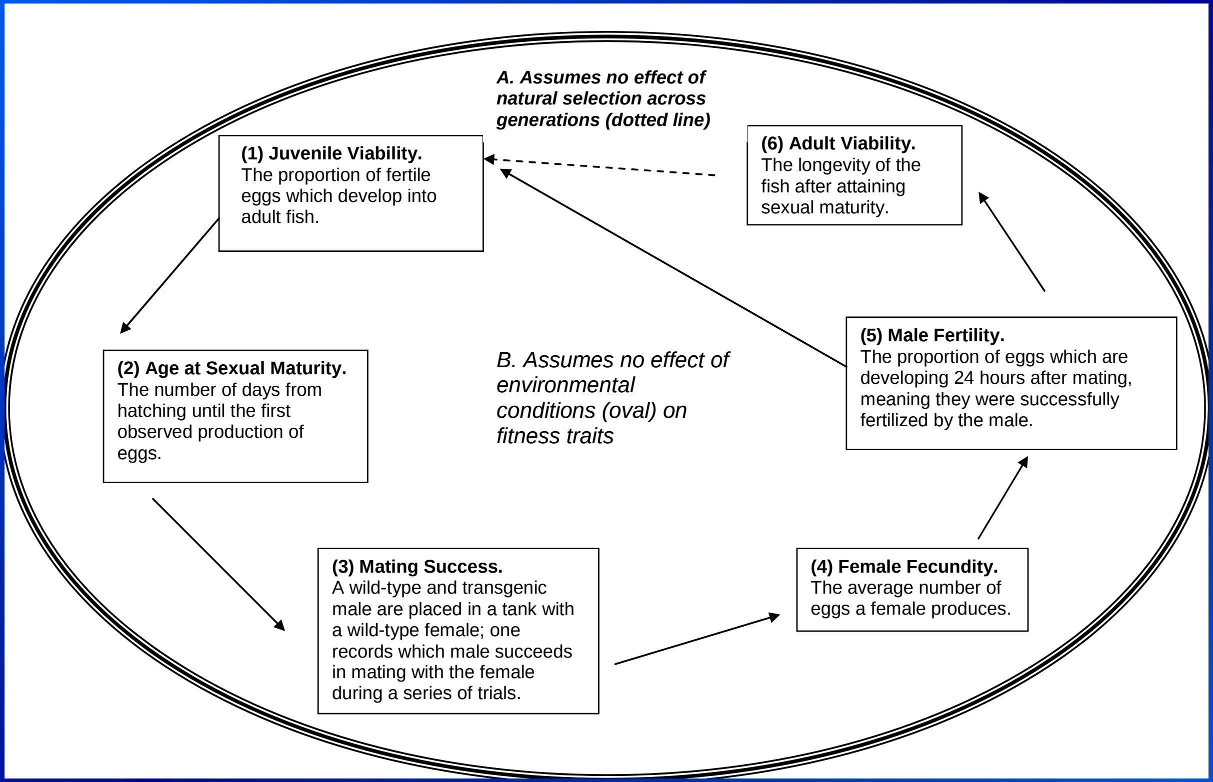
- *Other* transgenic lines may show:
- Cecropin, lactoferrin → heightened disease resistance
- Phytase → ability to efficiently utilize phytate
- …
- Or other traits that may increase fitness

What is the *fitness* of transgenic individuals?

- Trait-by-trait assessments of fitness do *not* address the integrated phenotype of an individual, the “target of selection”
- Especially if there are *tradeoffs* among fitness-related traits, how to predict the fate of the transgene in receiving populations (and hence, likelihood of harm)?
- → Consider effect of transgene expression on *net fitness* of individuals…

Net fitness model

(Muir, Howard and colleagues)

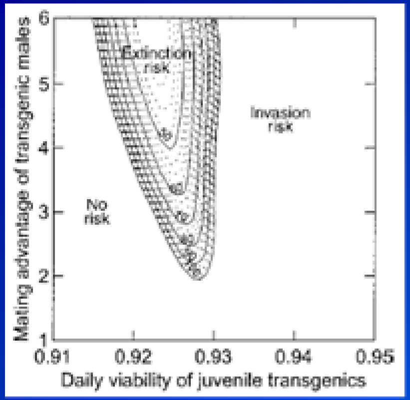


(Kapuscinski et al. 2007)

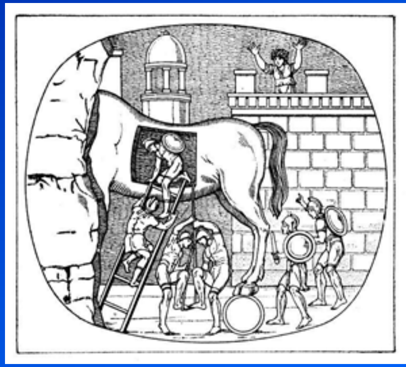
- Demographic model tracks transgene frequency, population size
- Predicts whether transgene will be lost or become more frequent

Net fitness model

- A key prediction: When a transgene effects certain fitness tradeoffs through the life cycle, (e.g., a gain in mating success at the cost of juvenile viability), the transgene could spread and threaten extinction
- Such tradeoffs are termed "Trojan gene effects"
- Others:
- Male mating success and reduced adult viability
- Increased adult viability and reduced male fertility
- Increased male mating success and adult viability,
- and reduced male fertility
- Similar predictions made by a more classical, selection-based model by Hedrick (2001)



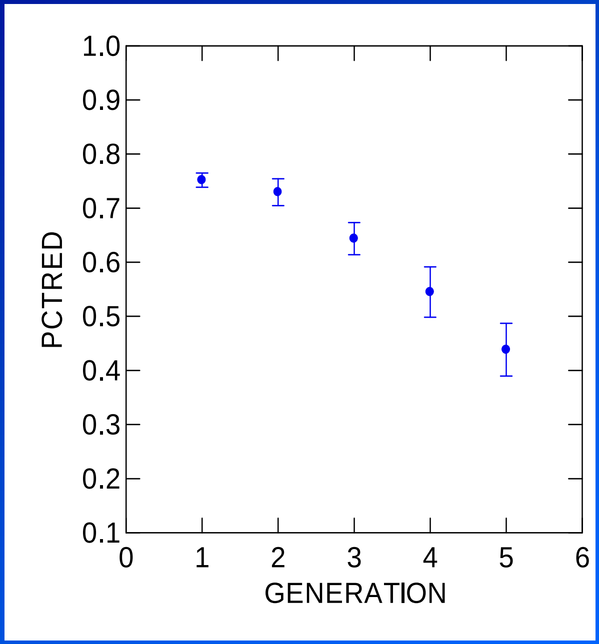
(Muir and Howard 2002)



The Trojan horse

Net fitness model

- Muir et al. measuring all six components of net fitness in red fluorescent zebrafish; elimination of transgene expected
- 20 replicate mesocosms, start with hemizygous fish (one copy of transgene; expect 75% fluorescent fish in first generation
- Frequency of transgene declined, now zero in three replicates
- Model works as expected, although stochastic processes result in variability about the predicted outcome



(Muir et al., unpublished)

Net fitness model

Limitations of net fitness modeling approach:

- Few data yet exist to parameterize and run the model
- Variances associated with predicted outcomes
- Data needed for *natural* conditions, esp. for limiting resource availability, fluctuating conditions → changing selective regimes
- Model does not account for introgression of transgene into varied genetic backgrounds
- Model does not account for selection at background loci that increases fitness of transgenics over time
- Area of active research!

Could interbreeding with wild populations pose genetic and evolutionary harms to receiving populations?

My assessment: Our ability to make quantitative predictions still rather limited – risk may generally be low, but it is non-zero.

Could heightened predation,
competition, or other processes
pose ecological harms
to receiving ecosystems?

Assessing ecological effects or transgenic fish a priori

(Devlin et al. 2007)

- Determine potential exposure of the ecosystem to transgenic fish
- Characterize the ecosystem
- Determine ecosystem resources and services used and contributed by transgenic fish
- Identify biotic and abiotic ecosystem components likely to interact with transgenic fish
- Define and prioritize potential harms
- Design experiments to assess phenotypic traits and critical environmental variables
- Identify factors contributing to uncertainty in empirical studies
- Predict ecological consequences from empirical studies

Possible ecological impacts

Key concerns:

- Competition with natural populations
- Predation upon natural populations

Competition with natural populations

- Six laboratory feeding trials of size-matched (250 g) coho salmon, one transgenic, one non-transgenic (Devlin et al. 1999)
- For first 3 contested pellets, transgenics consumed 2.5 times as many
- Overall transgenics consumed 2.9 times as many pellets
- GH transgene increases ability to compete for food
- Similar studies with Atlantic salmon, tilapia, common carp, …

Outcome of competition depends on food availability

- Food availability in nature is often limiting…
- Devlin et al. (2004) cohabited transgenic and non-transgenic coho salmon competing for different levels of food
- Transgenics outgrew non-transgenic fish, except when food availability was *high*
- When food availability was *low*, dominant individuals (usually transgenic) directed agonistic and cannibalistic behavior to other fish and dominated acquisition of food
- All groups containing transgenics crashed or went to extinction, while groups of non-transgenics exhibited 72$\pm$4% survival and population biomass increased
- Hence, effect of transgenics *differed with environmental conditions*
- Characteristics of receiving ecosystem affect assessment of ecological risk

Predation upon natural populations

Sundstrom et al. (2007) evaluated predation by transgenic and non-transgenic coho salmon upon fry prey in hatchery and naturalized stream environments

•*Under hatchery conditions*, transgenics grew dramatically larger than non-transgenics and exerted stronger predation effects, even after accounting for size difference

•*Under naturalized stream conditions*, trangenics grew only 20% larger than non-transgenics, and magnitude of difference in predation effects much reduced

Inferences:

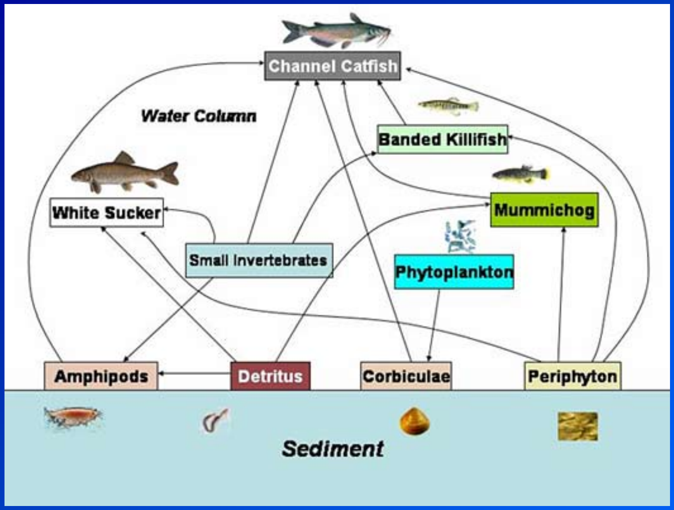
•Environment influences predation intensity

•Laboratory studies may overestimate predation risk

•Use of naturalized environments will be critical for obtaining reliable risk assessment data

Additional factors affecting assessment of ecological risks

- Impacts of predation can cascade through feeding webs – “top-down” effects
- Scale and frequency of introductions into a particular ecosystem will have large bearing on ecological risk
- Aquaculture escapees can outnumber wild fish in some ecosystems



An aquatic food web

Could heighted predation, competition, or other processes pose ecological harms to receiving ecosystems?

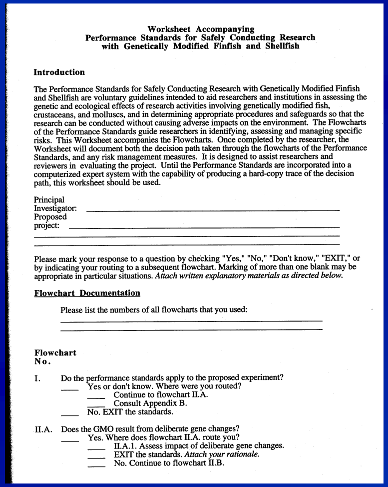
My assessment: Emerging picture – under a range of ecological conditions, there would be considerable risk of ecological harm becoming realized

Risk management → Risk assessment

- Recognizing that $R = P(E) \times P(H|E)$, R may be minimized by minimizing $P(E)$
- Ecological risk may be minimized by culturing transgenic fish under strict confinement:
- Onshore culture
- Recirculating aquaculture systems
- Reproductive confinement
- Effective operations management…

Risk Management

- Operations management:
- Ensure that culture activities promote confinement
- Prevent unauthorized human access
- Ensure inspection and maintenance
- No marketing of live fish



Worksheet Accompanying
Performance Standards for Safely Conducting Research
with Genetically Modified Finfish and Shellfish

Introduction

The Performance Standards for Safely Conducting Research with Genetically Modified Finfish and Shellfish are voluntary guidelines intended to aid researchers and institutions in assessing the genetic and ecological effects of research activities involving genetically modified fish, crustaceans, and molluscs, and in determining appropriate procedures and safeguards so that the research can be conducted without causing adverse impacts on the environment. The Flowcharts of the Performance Standards guide researchers in identifying, assessing and managing specific risks. This Worksheet accompanies the Flowcharts. Once completed by the researcher, the Worksheet will document both the decision path taken through the flowcharts of the Performance Standards, and any risk management measures. It is designed to assist researchers and reviewers in evaluating the project. Until the Performance Standards are incorporated into a computerized expert system with the capability of producing a hard-copy trace of the decision path, this worksheet should be used.

Principal Investigator: ____________________
Proposed project: ____________________

Please mark your response to a question by checking "Yes," "No," "Don't know," "EXIT," or by indicating your routing to a subsequent flowchart. Marking of more than one blank may be appropriate in particular situations. *Attach written explanatory materials as directed below.*

Flowchart Documentation

Please list the numbers of all flowcharts that you used:

Flowchart No.

I. Do the performance standards apply to the proposed experiment?
- ____ Yes or don't know. Where were you routed?
  - ____ Continue to flowchart II.A.
  - ____ Consult Appendix B.
- ____ No. EXIT the standards.

II.A. Does the GMO result from deliberate gene changes?
- ____ Yes. Where does flowchart II.A. route you?
  - ____ II.A.1. Assess impact of deliberate gene changes.
  - ____ EXIT the standards. *Attach your rationale.*
  - ____ No. Continue to flowchart II.B.

State of ecological risk assessment for transgenic fish

Risk assessment:

•Development of quantitative risk assessment is incomplete, esp. regarding likelihood of harm given exposure to hazard

•Need more studies quantifying net fitness, especially under (near-)wild conditions

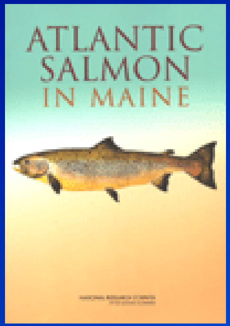
•Need advances in understanding of certain fundamental issues, e.g., likelihood of outbreeding depression, GxE effects, ecological interactions in wild

Risk management:

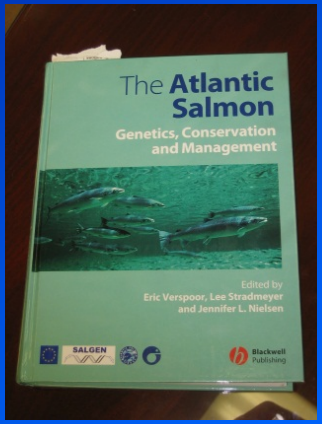
•Need to demonstrate effectiveness and economic viability of aquaculture production under confinement conditions

Sources of Useful Information

- National Research Council: *Atlantic salmon in Maine*. www.nap.edu.



- Verspoor, E. et al.:
  The Atlantic salmon: Genetics, Conservation, and Management. Blackwell Publishing.



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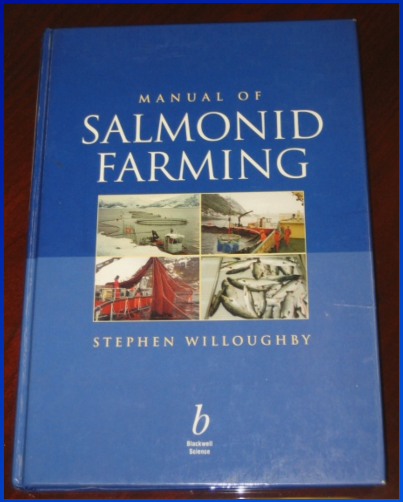

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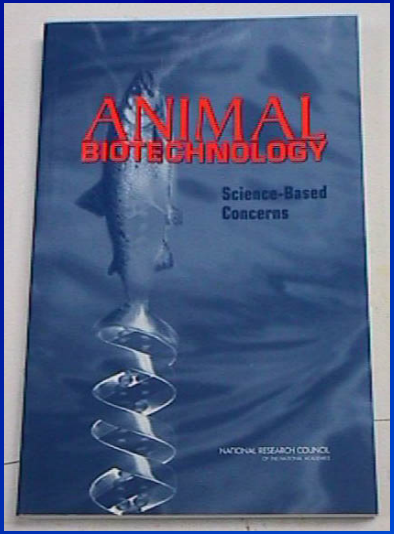

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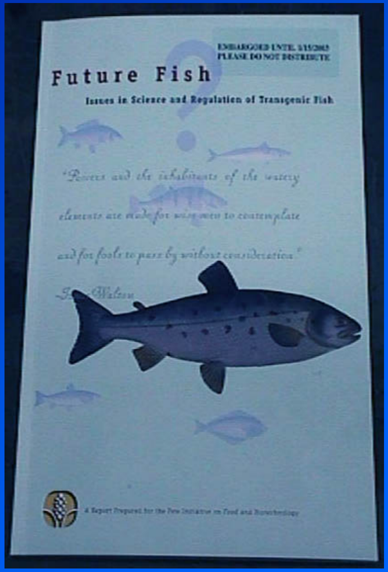

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